

person has recently fired a gun. This service is currently provided by the Harris County Medical Examiner's Lab.⁹⁹

We also noted that the Firearms Section performed trigger pull determinations on every firearm submitted to the Crime Lab. The time spent on these determinations would, in our view, be better used comparing ammunition components received within the chambers of submitted firearms and, where appropriate, performing muzzle-to-target distance determinations. Where there is no issue regarding unintentional firing, an examiner does not gain useful data by conducting a trigger pull examination. Furthermore, in the course of test firing, an examiner can readily recognize weapons that appear to have extremely light trigger pulls. We believe that examiners' time would be better spent if trigger pull examinations are performed only where there is a question regarding unintentional firing or where the test firing identifies a light trigger pull.

Overall, most cases in the Firearms Section were properly examined and reported in a timely manner. Although our review has not uncovered any major issues in this section, we note that failing to strictly follow generally accepted laboratory practices creates a risk for potentially serious errors. We include in this category failing to document thoroughly each step of an examination, allocate time in a manner that yields the most useful information, and report inconclusive findings when the results merit.

VII. Toxicology

Forensic toxicology involves the detection, quantitation, and identification of potential toxins, including drugs and alcohol, in bodily fluids and tissues. Two basic steps are normally involved: (1) initial screening tests and (2) confirmatory tests. During the screening step, lab analysts test for the presence of a wide range of drugs or other toxins. Screening tests are not necessarily specific for a particular toxin; thus, until initial results are confirmed, they are viewed as tentative at best. Confirmatory tests reduce the risk of false positive test results, which can occasionally occur when a substance's chemical structure is similar to that of another substance or when a contaminant has been introduced. At the Crime Lab, screening tests have been commonly performed

⁹⁹ The examiners in the Firearms Section also do not currently perform toolmark examinations, which, among other things, involve comparisons of pry marks, hammer head impressions, and other evidence related to crimes such as burglaries. This is a service commonly provided by forensic firearms laboratories.

using fluorescence polarization immunoassay ("FPIA") techniques and thin-layer chromatography ("TLC"). GC/MS testing was normally used by the Toxicology Section as a confirmatory test.

Until October 2003, most of the toxicology analyses performed at the Crime Lab involved the analysis of blood and urine samples. Blood and, more frequently, urine was typically analyzed for alcohol and other drugs of abuse. These specimens were usually collected from individuals suspected of driving under the influence of alcohol and other drugs. Quantitation was not performed, except for cases involving alcohol.

As was discussed in greater detail in our Phase I reports, questions regarding the performance of the Toxicology Section were raised after the Criminalist IV supervisor of the section, Pauline Louie, failed a competency test in October 2003. This development ultimately led to the suspension of toxicology analysis by the Crime Lab in October 2003.

In May 2005, the Crime Lab was accredited by ASCLD/LAB to perform blood alcohol analysis, and its toxicology case work is now limited to this area. Three analysts currently perform blood alcohol analysis.¹⁰⁰ These three analysts are also responsible for calibrating and maintaining HPD breath alcohol analysis equipment. Analysts in the Crime Lab do not administer breath tests to suspects but do provide training to the HPD officers who do.

A. Testing Procedures Used by the Toxicology Section

The same fundamental antigen-antibody reactions that apply in serology can be used for detecting drugs in blood and urine. The Toxicology Section used an FDA-approved FPIA technique as a screening test. FPIA involves the use of a drug antigen that is created with a fluorescent "label." When these antibodies are added to a blood or urine specimen containing the drug antigens, the antigens in the sample move to attach themselves to the antibodies. The sample is exposed to light, and this movement creates measurable changes in the intensity of the light. The changes are proportional to the quantity of drug antigen present in the specimen being tested. Again, chemically similar substances can create false positive results when immunoassay techniques are used, so confirmatory tests are required.

¹⁰⁰ A supervisory position for the Toxicology Section is currently unfilled, and there are no immediate plans to fill it.

Like many other forensic and clinical laboratories, the Crime Lab used a commercially available thin-layer chromatography ("TLC") system to test urine samples for drugs. TLC is used to separate components of a mixture and to tentatively identify those components. The varying colors, shapes, and *Rf* values¹⁰¹ observed on the resulting chromatogram are compared with patterns produced by known compounds to make a tentative identification of substances present in the sample.

B. Proficiency Testing

The preliminary results of our Phase I investigation indicated that no toxicology proficiency testing was performed between 1995 and 1999. Since then, we have received additional proficiency test files indicating that the toxicology proficiency testing hiatus was for a slightly shorter period -- between late 1995 and early 1998. In addition, the Crime Lab's subscription to a bi-annual Department of Transportation blood alcohol proficiency testing program lapsed at some point during this time, and we have seen no blood alcohol proficiency test results since then. Aside from the period noted, drug proficiency testing was performed at the rate of five to six times per year. Thirty-three proficiency tests performed by Toxicology Section analysts between 1998 and 2003 have been identified and reviewed.

Test results were generally good and sometimes excellent. Most of the tests were examined by more than one analyst and were then reviewed by the section supervisor. However, three tests performed during the review period yielded false positive results, *i.e.*, the Crime Lab analysts incorrectly reported drugs that were not actually present in the test sample. In one case, three analysts noted "indications" of methamphetamine in their work notes, but none of the analysts attempted to confirm the identification. The sample did contain a drug with a chemical structure similar to methamphetamine. In another test, two analysts incorrectly reported the presence of methorphan (a codeine-based cough suppressant), but failed to identify cimetidine that was present in the sample.¹⁰²

¹⁰¹ *Rf* value is expressed in terms of a ratio, in which the distance traveled by the substance being tested is compared with the distance traveled by the transporting solvent.

¹⁰² Cimetidine is commonly prescribed for the treatment of gastric reflux disease. It is not a drug of abuse and, therefore, is not the type of drug typically tested for in DUI cases.

A third false positive involved the identification of a narcotic metabolite by the section supervisor. For a period of time beginning around 2000, the Crime Lab experienced a high rate of turnover and was understaffed. As a result, Ms. Louie, the Criminalist IV supervisor over both the Toxicology and Controlled Substances Sections, returned to bench work in toxicology after a relatively long absence from bench case work. This work was in addition to her many supervisory duties and frequent court appearances. Because of her dual role as analyst and supervisor, there was no meaningful oversight of Ms. Louie's own performance on proficiency tests during this time. Nevertheless, to her credit, she rated her own performance on this proficiency test as unsatisfactory.¹⁰³

In a number of other tests, Toxicology Section analysts failed to identify substances that actually were present in the sample. One was a cannabinoid commonly encountered in forensic toxicology. For no apparent reason, the analysts simply did not perform tests that would have identified it. In another proficiency test, the analysts correctly identified cannabinoids, but did so based on a positive FPIA and inadequate GC/MS test results.¹⁰⁴

C. Results of Toxicology Case Reviews

Toxicology case files have been selected from a total universe of 1,555 toxicology cases handled by the Crime Lab between 1998 and 2004. We recalibrated our original toxicology sample after we discovered a significant number of the cases in the sample actually involved analysis by other sections in the Crime Lab, particularly the Controlled Substances Section. The recalibrated toxicology sample includes 308 cases, 94 (31%) of which we have reviewed.

To date, we have identified only one toxicology case as potentially involving a major issue relating to the reliability of the work performed. In that case the analyst concluded -- on the basis of GC/MS testing alone -- that a blood

¹⁰³ Additionally, a fourth false positive test result occurred in a proficiency test administered in 1997 (outside the 1998-2004 period of our formal review). Toxicology Section analysts identified the presence of cocaine in the sample. The sample did contain a cocaine metabolite, but the test provider firmly denied that the sample contained any cocaine, and most labs participating in the test did not report it. The Crime Lab's analytical data clearly shows cocaine, and the most reasonable explanation for this is sample contamination at some stage in the examination process.

¹⁰⁴ In that same test, the analysts failed to identify the presence of erythromycin, a drug that, similar to cimetidine, is not typically tested for in DUI cases.

sample was positive for heroin, cocaine, and PCP. The GC/MS data were, in our view, interpreted correctly. However, no morphine was identified by the analyst. Because heroin is almost immediately metabolized into morphine when it enters the human body, a positive heroin test without the presence of morphine is an unlikely pharmacological result and could indicate possible sample contamination. In light of the pharmacologically questionable result and the absence of a second test, we consider the work in this case to be inconsistent with generally accepted forensic science practices.

A number of other case files reviewed thus far involved drug identifications that were based on a single test, usually GC/MS. In some, the analytical data were not interpreted as rigorously as they might have been. For example, mass spectrometer "matches" were identified by the analysts but were not, in our view, strong matches. It appears that analysts may have treated the GC retention time in a GC/MS run as a second, confirmatory test, even though it is not an independent test. Another common issue in many of these cases was the lack of technical review by a qualified person other than the analyst who had performed the work. This typically occurred when the section supervisor was the only person performing toxicological analysis at the Crime Lab.

We observed deficiencies in the identification of some drugs and metabolites in some toxicology cases. However, in each of these cases, other drugs and metabolites were properly identified and correctly reported. As a result, because other controlled substances were detected in the samples, we concluded that the failure to identify the additional drugs or metabolites in the sample probably did not have any effect on the outcome of the case.

D. Blood and Urine Alcohol Testing

There were no significant issues identified in the blood and urine alcohol cases reviewed thus far. Moreover, our preliminary review of the Toxicology Section's work indicates that there has been continual improvement in procedures and documentation in this area. As of 2004, the procedures used by the Crime Lab were state of the art.

The files reviewed to date indicate that, with a few exceptions, the work performed by the Toxicology Section has been satisfactory. Between 1998 and 2004, there was an obvious and demonstrable improvement in the analytical procedures and processes used by the section. Toxicology case files are well organized, the reviews are properly documented (except as noted above), and an

appropriate range of analytical procedures has been performed in most of the cases reviewed.

VIII. Questioned Documents

In the forensic context, document examination serves the goal of determining how or by whom a document was generated. Document examiners attempt to establish the date, source, history, preparation, authenticity, and relationship of documents. Their work involves any or all of the following:

- identifying or eliminating the source of handwriting by comparison of unknowns with knowns;
- identifying or eliminating the source of typewriting and the output of other mechanical or electronic imaging devices;
- comparing or identifying inks and papers;
- visualizing indented marks; and
- restoring altered, damaged, and erased writing or text.

To perform that work, document examiners must have the skills necessary to successfully conduct macroscopic and microscopic examinations, spectral analysis with infrared/UV instrumentation, and electrostatic imaging of latent impressions, supplemented by the knowledge and experience necessary to properly interpret the results. In addition, document examiners must be able to write reports, explain the testing process and their findings to investigators, and testify in hearings and trials.

One example of document examination is the identification or elimination of a person as the source of handwriting on documents in many different types of cases. Results of such comparisons may range from positive identification of the writer to definite elimination, with differing intermediate probabilities including inability to identify or eliminate.

To identify someone as the source of handwriting, a document examiner needs contemporaneous samples of known writing from the suspected writer to compare with the questioned writing. When comparing the two writings, the examiner looks at a variety of characteristics including such things as the formation and proportions of the letter and letter combinations, strokes, pen pressure variations, and pen lifts in order to find repetitive handwriting habits in

both writings. The examiner also looks for significant differences that could possibly eliminate the suspect as the source of the writing.

A. Overview of the Questioned Documents Section

Document examination work was conducted in HPD's Identification Division until the mid-1980s. Following a number of employee departures, the Questioned Documents Section was closed. It did not re-open until the current examiner, Randy Carodine, finished his three-year training with outside experts in 1999. Re-establishing the Questioned Documents Section was a challenging task. To meet that challenge, Mr. Carodine solicited advice from a qualified document examiner in Houston. To develop interest among HPD investigators, he distributed a circular within HPD and visited each division in HPD to distribute pamphlets that detailed the Questioned Documents Section's capabilities and described how to submit evidence properly for examination.

In 2004, when the Crime Lab began its accreditation application process, the Questioned Documents Section was transferred from the Identification Division and became part of the Crime Lab. This transfer was completed in anticipation of a Texas state law that became effective in 2005, which provides that forensic science evidence can be admitted in courts only if the lab is accredited. Although Mr. Carodine tried for some time to implement formalized procedures within the Questioned Documents Section, he did not receive authorization to enact those procedures until the section was transferred to the Crime Lab. The Questioned Documents Section was then finally able to develop and implement detailed SOPs.

Based on the register of cases handled by the questioned documents examiner, we originally estimated that we would review about 200 cases, encompassing all of the questioned documents cases from 1998 through 2004. After we began reviewing the case files, however, we determined that only 91 of these cases actually involved work performed by the questioned documents examiner. We have completed our review of all 91 of these cases, and we have identified none as involving a major issue.

Overall, we were impressed with Mr. Carodine's knowledge and the quality of his work. In fact, the vast majority of cases were well documented, with impressive notes that supported the conclusions reported. We did not disagree with any of the results or opinions expressed by Mr. Carodine. We did note some minor issues, which mainly revolved around two aspects of the

Questioned Documents Section's work -- the issuance of reports and the performance of technical reviews.

Most of the minor issues we noted occurred before the implementation of more detailed and specific SOPs in 2004. Before those SOPs were adopted, the Questioned Documents Section relied on a four-page SOP. Although it was not specific in most areas, the pre-2004 SOPs did require that a report be issued for all cases. At times, Mr. Carodine placed case numbers in a log but did not create reports documenting his work on those cases. Whenever Mr. Carodine gave advice or an investigative lead to an investigator over the telephone, he established a case number in the log. For example, when investigators and district attorneys telephoned to ask Mr. Carodine what was necessary to submit a case for examination or whether a particular examination was possible, he logged those inquiries to document the fact that he gave out technical advice. However, Mr. Carodine did not create a report documenting his advice. We believe that when a case number is established -- especially if any work is performed on a case -- questioned document examiners should track the evidence, prepare notes, and prepare a report on that case.

We also observed that some of the Questioned Documents Section's casework had not undergone a technical review, which is a review by another qualified person of the examiner's notes, data, and other documentation supporting the examiner's conclusions. Since the Lab's Questioned Documents Section has had only one examiner since its re-opening in 1999, that examiner was forced to develop a technical review network for his examinations. Even though none of Mr. Carodine's supervisors within the Identification Division required a technical review of his work, he believed it was necessary and took the initiative to ask experts outside HPD to perform such reviews. In most of the cases where definitive opinions, such as the elimination or identification of a source of writing, were given, Mr. Carodine had his work independently reviewed.

Mr. Carodine told us, however, that he did not usually seek verifications on less-definitive results (when, for example, he could not come to a conclusion or only reported an indication). At other times when he did have his work reviewed, he did not document this in the case file because the independent reviewer did not wish to risk a possible subpoena and, hence, did not want to be identified in the Crime Lab's case file. Because the Questioned Documents Section is a one-person unit, special and sometimes cumbersome arrangements had to be made for technical reviews. Since 2004, the section has had a formalized technical review process in place.

B. The Questioned Document Section's Workload

We were somewhat surprised by the Questioned Documents Section's relatively small workload -- 91 cases in about five years. In light of the size of the City of Houston and the HPD, we would have expected the Questioned Documents Section's workload to be much greater. The examiner's efforts to promote the re-opened Questioned Documents Section did initially result in more cases being submitted, but the section has been continuously underutilized for a number of years.¹⁰⁵

There are a few possible explanations for the underutilization of the Questioned Documents Section. One may be that investigators are unaware that the Crime Lab has an operating Questioned Documents Section. Another possibility may be that they are unaware of how document examination can assist their investigations. Yet another possibility is that investigators have become discouraged after submitting evidence to the section and receiving inconclusive results.¹⁰⁶ Whatever the reasons, HPD is not fully utilizing its highly competent document examiner.

¹⁰⁵ In fact, Mr. Carodine was given additional tasks from the Identification Division in order to fill his time during the 1998-2004 period.

¹⁰⁶ This is a common (and misguided) complaint by investigators about document examination. Questioned documents examiners will often be unable to make a conclusive determination because inadequate specimens are submitted for examination. For example, the examiner cannot make a conclusive finding when he is working with a bad photocopy of a document.

Conclusion

This report summarizes the case reviews we have conducted in Phase II of our investigation from September 2005 through the first week of December 2005. Thanks to the cooperation provided by HPD and the sustained hard work by members of our investigative team, we have completed over 1,100 substantive case reviews out of our sample of approximately 2,700. More specifically, over the past three months, we have completed a significant percentage of case reviews in DNA and serology for cases handled from 1987 through 2002 and in all of the other areas of forensic science in which the Crime Lab performed work during the period 1998 through 2004.

As revealed by the case reviews, and as described in great detail in the body of this report, the record is mixed. We have observed some excellent work performed by Crime Lab analysts and examiners, especially in the Toxicology, Firearms, and Questioned Documents Sections of the Crime Lab. In some sections, such as Controlled Substances and Trace Evidence, the record is more balanced: We have noted some fine work performed, but we have also identified a number of significant deficiencies.

Unfortunately, our reviews of cases involving serology and DNA analysis have shown a near total breakdown in the forensic science function in those two important sections for at least a 15-year period from 1987 through 2002. Already, we have seen a disturbing and pervasive pattern involving repeated failures to report results of scientific testing, including results that were exculpatory of the suspect; the general failure to use appropriate scientific controls to ensure the reliability of reported results; the failure to properly calculate and communicate the meaning of statistics in scientific reports and courtroom testimony in order to accurately convey the significance of test findings; and the absence of any meaningful internal or external oversight of the critical work performed by serology and DNA analysts. Our work to date in reviewing cases analyzed by these sections reflects a level of performance completely unacceptable in a forensic science laboratory providing critical support to the criminal justice system.

We still have considerable work to do in completing the case reviews as well as in conducting further interviews and gathering the additional information necessary to come to final conclusions about the problems we have identified to date. The remaining case reviews and additional investigative work will provide us with an even stronger foundation on which to base

recommendations for the Crime Lab, which is a central element of our mandate. Once the case reviews and further investigation have been completed, we will not only have a full and accurate picture of the past problems in the Crime Lab -- their scope and their causes -- but also a detailed body of knowledge that can serve as the basis for improving the quality of the Crime Lab's work and enhancing its contribution to the criminal justice system.



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January 4, 2006

Appendix A

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Appendix B

Discussion of Serology Techniques Used by the Crime Lab

During the 1980s and early 1990s, forensic serology practiced in the Crime Lab primarily involved ABO typing. The discipline of forensic serology at the time also included procedures for the identification of biochemical genetic markers in blood such as certain enzymes and proteins, but it appears that the Crime Lab rarely used such testing results to associate or disassociate stains with particular individuals.¹ Although we found Crime Lab log books recording the results of electrophoretic runs associated with enzyme testing and have seen Lab notes and worksheets in case files reflecting that enzyme testing was performed in certain cases, Lab serologists rarely reported results obtained through enzyme testing. Thus far, we have identified virtually no cases in which the Crime Lab reported the results of enzyme testing for use in an investigation or prosecution. Accordingly, this discussion provides an overview only of techniques related to ABO testing commonly used by serologists in the Crime Lab.

I. ABO and Lewis Testing of Known Reference Standards

"Known reference standards" are samples of blood and saliva collected from persons potentially associated with evidence stains. For example, known reference samples are used to compare a suspect's genetic characteristics, such as blood type, with the genetic characteristics of blood or secretion stains recovered from a crime scene. This process is followed in order to develop evidence tending to show that the suspect is included -- or excluded -- as a possible source of biological evidence. Known reference standards are most commonly collected from suspects in the form of a tube of blood drawn from the arm and saliva collected by a swabbing of the lining of the cheek with cotton.

Crime laboratories typically subject known blood reference standards to both ABO testing, in order to determine the suspect's ABO blood type, and

¹ An enzyme is a type of protein that acts as a catalyst for certain specific biochemical reactions. Historically, forensic scientists have been particularly interested in certain enzymes and other proteins found in blood -- such as PGM (phosphoglucomutase), EAP (erythrocyte acid phosphatase), EsD (esterase D1), Hp (haptoglobin), and others -- because those enzymes and proteins are "polymorphic," meaning they exist in different forms and, therefore, are useful in distinguishing between individuals. The various inherited forms of these polymorphic enzymes and proteins are called "alleles." The analysis of such enzymes and proteins involves the separation of the alleles through a process known as electrophoresis.

"Lewis" testing, which is helpful to predict or confirm whether the suspect can be expected to be a secretor whose ABO type can be detected in other bodily fluids such as semen. ABO and Lewis testing of the known reference blood samples are normally conducted by testing the antigens on the red blood cells in the reference samples with commercially-available "antisera."² This is commonly referred to as "direct" testing because the subject's red cells are tested directly by combining known antibodies with the red cells in a test well and observing the test result. A positive test result is manifested by agglutination (clumping) of the cells caused by the binding of the red cell antigens to the antibodies in the test reagent.³ Such agglutination is clearly observable with the naked eye or under low magnification, and the absence of clumping indicates the absence of the antigen being tested for. Thus, for example, if agglutination is observed in the test well containing anti-A antibodies, then the serologist would record a positive result for the presence of type A activity.

Known reference saliva standards typically are collected from a suspect on sterile cotton swabs or sterile gauze and allowed to air dry to preserve the sample from mold or decomposition caused by bacteria. The purpose of the collection of a known reference saliva standard is to enable the forensic serologist to determine the subject's ABO secretor status. The method used for testing the dried reference saliva standard is called ABO absorption inhibition ("AI"), and is the same method that is used for testing for ABO factors present in secretion stain evidence. The AI testing technique is described briefly below.

II. ABO Testing of Bloodstains by Absorption Elution

Absorption elution ("AE") is the generally accepted forensic serology testing method for determining the ABO factors located in bloodstain evidence. AE is also considered to be a type of "direct" testing. AE involves testing the ABO antigens in bloodstains directly by adding commercially-available known ABO antibodies to the bloodstained material, permitting the antibodies to bind to the ABO antigens in the bloodstain, and then eluting those antibodies from the

² Antisera are solutions containing specific antibodies.

³ A "reagent" is a substance used in a chemical reaction to examine or produce other substances. In the context of ABO testing, for example, it is the solution containing antigens or antibodies the reaction of which the scientist is observing in order make a blood type determination.

bloodstains.⁴ The ABO typing is then performed by adding red cells of known ABO type into test wells containing the eluted ABO antibodies obtained from the bloodstain and observing the agglutination that signals a positive test result for the presence of the corresponding ABO antigens.

Occasionally, blood crusts or evidence items with bloodstains on hard surfaces are submitted to a crime lab for testing. When that occurs, a serologist transfers the bloodstain to clean cotton threads by dissolving the bloodstain and then allowing the concentrated solution of blood to dry onto the cotton threads. AE can then be conducted on the cotton threads bearing the transferred bloodstains.⁵

III. ABO Testing of Secretion Stains by Absorption Inhibition

AI is the generally accepted forensic testing method for determining the ABO factors present in stains related to bodily fluids such as semen, saliva, vaginal secretions, perspiration, tears, nasal mucous, or mixtures of these fluids. The same AI method is also used to test the known reference saliva standards obtained from a suspect to determine whether he or she is a "secretor" -- *i.e.*, a person whose ABO type is expressed in his or her bodily fluid secretions.⁶

⁴ The term "elution" refers to the immunological process of freeing (*i.e.*, eluting) bound antibodies contained in bloodstain evidence from the bloodstains by applying heat to break the antigen-antibody bonds.

⁵ Sometimes AE testing of bloodstains was conducted in conjunction with a form of "reverse" blood typing called the "Lattes Crust" test, named after Leon Lattes who developed this technique. Forensic serology laboratories commonly used Lattes testing to obtain ABO typing results from scrapings of dried blood crust collected from hard surfaces, such as glass or a weapon (hence the term "Lattes Crust" test). The Lattes Crust test, however, is less sensitive than AE. Consequently, more bloodstain material must be consumed to conduct a Lattes Crust test than the amount of bloodstain material needed for AE. After other genetic marker systems (polymorphic enzymes and proteins) became available to forensic serologists in the early to mid-1970s, the use of Lattes Crust testing as an adjunct to AE for ABO typing of bloodstains competed with the other genetic markers for the consumption of limited amounts of bloodstain material and generally fell out of favor in crime laboratories. AE was sufficiently sensitive and accurate to be relied upon for good quality ABO typing of bloodstains without the parallel use of Lattes Crust testing.

⁶ Bloodstains are not tested by the AI method because, among other things, the concentration of ABO factors in bloodstains is significantly less than the concentration of ABO factors found in secretion stains. Consequently, the forensic serologists typically use the direct AE method, which is more sensitive than AI, to test bloodstains.

AI is an "indirect" ABO test method, meaning that the presence of an ABO factor in a secretion evidence stain is determined by observation of a diminished level or absence of agglutination in the test solution.⁷ Cuttings from secretion stains (such as from stained underwear, a vaginal swab obtained from a rape kit, or saliva stained cigarette filter paper) are placed into three separate tubes. A small volume of test reagent containing a pre-determined dilution of the appropriate antibody is added to each tube to enable the antibody to incubate with the secretion stain. Each tube contains one of three antibody dilutions -- either anti-A, anti-B, or "anti-O."⁸

If the corresponding antigen is present in the questioned stain, the strength of the antibody remaining in the solution will be diminished as the antibody becomes bound to the corresponding antigen by forming an antigen-antibody complex. The serologist then removes the residual antibody solution from each tube and places these residual solutions on a glass plate or glass slide, which is tested with a freshly prepared suspension of the corresponding commercially-available, known ABO cells. The three residual solutions are mixed with known ABO type A cells, type B cells, and type O cells. Because AI is a form of "reverse" testing, the presence of agglutination in the test slide for a particular antigen indicates that the corresponding ABO factor is *not* present in the secretion stain being tested. For example, the absence of agglutination in the antibody solution mixed with A cells indicates the presence of type A activity in the secretion sample.

⁷ By contrast, presence of a specific ABO factor as a result of a "direct" testing method such as AE is indicated by observation of agglutination in a test well.

⁸ Because there is no common human antibody against ABO type O blood cells, scientists use an extract from gorse seeds, *Ulex Europeus*, to cause type O cells to agglutinate. The seed extract, called "lectin," agglutinates the H antigen found on all ABO cells, but the agglutination occurs in much higher concentration in the presence of type O cells. Thus, the term "anti-H" seed lectin has become synonymous with "anti-O" for purposes of ABO testing.

Appendix C

Discussion of DNA Profiling Technology and Techniques Used by the Crime Lab

I. RFLP Testing

Testing of restriction fragment length polymorphisms ("RFLP") involves the analysis of DNA fragments that are produced by using restriction enzymes, which act like scissors to cut DNA into fragments at specific locations along the chromosome. These DNA fragments of different lengths, also known as alleles, are distinguishable from each other in human populations. Hence, the genetic variation that RFLP identifies is known as "length polymorphism."¹

Once DNA molecules have been cut into pieces by restriction enzymes, the resulting fragment lengths are separated through a process known as gel electrophoresis.² During the electrophoretic process, DNA fragments migrate through a gel, with the smaller DNA fragments moving at a faster rate than the larger DNA fragments. It is the results of this migration process that enable the forensic DNA analyst to distinguish between fragment sizes of DNA.

In order to generate reliable RFLP test results, the loading of DNA samples into the electrophoresis gel must be performed correctly. The DNA analyst must deliver the solution containing DNA samples into the appropriate hole (or well) located on the gel-coated plate. To accomplish this, the tip of the pipette containing the DNA sample must be lowered into the buffer solution in which the gel is submerged and the DNA sample must be ejected above the well in order to permit the DNA extract to flow into the well. Because each well has a limited capacity, the DNA analyst must take care not to overfill it. This precise process of loading of DNA samples requires training, patience, and skill to avoid contamination as a result of crossover of the DNA extract into one or more adjacent wells.

¹ Another form of forensic DNA testing that involves the identification of genetic variation attributable to length polymorphism is the testing for short tandem repeats ("STRs") of DNA markers. By contrast, other types of DNA testing are used to detect differences in individual nucleotides or base pairs, rather than DNA fragment length, which is a form of genetic variation known as "single-nucleotide polymorphism" or "SNP." PCR-based DQ-alpha and Polymarker testing, discussed below, are examples of SNPs that have been used in forensic applications.

² Electrophoresis is a technique that separates molecules based on their size and electrical charge.

In order to avoid the potential for such contamination, which might cause a false positive typing result, the DNA analyst must avoid placing DNA extracted from evidence in a well immediately adjacent to a DNA sample extracted from a known reference sample taken from a victim or a suspect. To reduce the risk of crossover contamination and the potential for a false positive resulting from a mistake in the gel-loading process, the best practice is for the analyst to leave an empty well between a questioned sample and a known reference sample. In this way, any appearance of a DNA profile in the empty lane (or control lane) between a question sample and the reference sample will signal that contamination has occurred and that the analyst must take appropriate remedial action.

Once the electrophoretic process is complete, the DNA analyst transfers the separated DNA fragments from the electrophoresis gel to a permeable nylon membrane through a technique known as "Southern blotting." The DNA fragments are then chemically bound to the membrane to allow the results of the electrophoretic separation of the fragments to be visualized through a radioactive or chemiluminescent process.

To visualize the patterns generated by the electrophoresis process, DNA fragments bound to the nylon membrane are made radioactive or chemically active through the use of commercially-available "probes," a process known as "hybridization."³ The membranes are then placed in close contact with x-ray film, which is exposed at very cold temperatures for periods ranging from hours to weeks. The analyst then develops the x-ray film in order to reveal the images of the radioactive or chemically labeled DNA allele fragments. These x-ray film images, known as "autoradiographs" or "autorads," appear as clear films with dark bands on them. The DNA analyst determines the size of each band by comparison with known sizing standards. The size of the evidence fragments are compared to those in the known reference samples.

Sometimes the banding patterns can appear too faintly for reliable interpretation of the RFLP alleles. When that occurs, the analyst must take steps to try to enhance those results. The best practice for enhancing results is to

³ These radioactively or chemically labeled probes are fragments of DNA of known molecular structure and contain a base sequence complementary to the RFLPs being identified.

expose a second film for a longer period of time. This allows the analyst to interpret faint bands with higher confidence.⁴

II. Early PCR-Based Testing

The PCR process, developed by Dr. Kerry Mullis in the mid-1980s, revolutionized molecular biology by providing scientists the ability to replicate (or amplify) extremely small amounts of DNA up to a billion-fold. PCR's impact on forensic DNA analysis was particularly significant because it enabled forensic scientists to obtain meaningful results from evidentiary samples of DNA that would have been previously too degraded or too low in quantity for successful RFLP testing. PCR-based testing also has the advantage of providing a much faster turnaround time than RFLP testing. A forensic DNA laboratory can complete most PCR testing in a matter of days -- a significant improvement over the weeks or months it could take to complete RFLP testing.

One drawback of forensic PCR technology is that it is extremely susceptible to contamination by DNA from other sources, including other items of evidence, the investigators who collected the evidence, and the forensic DNA analysts themselves. Although the use of proper standards and controls can usually signal any contamination that occurs in the laboratory, proper training, compliance with strict quality control procedures, and diligence are required to avoid contamination during the analysis of forensic samples. This underscores the importance of strict compliance with proper standard operating procedures, adequate training, close supervision, and mandatory standards and controls in order to obtain accurate and reliable DNA test results.

PCR technology is a patented process that is very closely regulated through licenses from the patent holders. Consequently, virtually all of the test reagent kits used in crime laboratories are sold by a limited number of vendors that confer the licensing rights to use PCR for forensic applications with the purchase of the kits. The advantage is that high quality kits with consistently high performance characteristics are available to all forensic laboratories. This allows standardization of the loci, the kits, and the procedures across the entire forensic DNA testing community.

⁴ It is important to note that stripping the membrane of probes and re-hybridizing with fresh probes eventually will lead to a reduction in the amount of DNA bound to the membrane.

A. DQ Alpha

DQ Alpha (also known as "DQ α " and later "DQA1") refers to a gene located in the human leukocyte antigen ("HLA") complex on the short arm of the sixth chromosome in humans. In the late 1980s, the AmpliType™ HLA DQ α Forensic DNA Amplification and Typing Kit was introduced to forensic laboratories. The kit distinguished six alleles or genetic variants at the DQA1 locus, which defined a total of 21 different genotypes. The kit format was known as "reverse dot blot" that came in the form of a probe strip containing a series of test dots. The actual DQ α typing test procedure involves three stages:

- (1) DNA extraction;
- (2) DNA amplification through the PCR process; and
- (3) DNA typing, which includes de-naturation of the amplified DQ α product, hybridization of the evidentiary and known reference DNA samples to probe strips, stringent washing of the probe strips in solution, and, finally, interpretation of the color development appearing in the dots contained on the test strip.

Specifically, DNA probes are immobilized onto a nylon typing strip in a pattern of a series of dots. During the hybridization step, amplified DQ α DNA from the test samples is captured by these probes and retained on the typing strip. During the stringent washing stage of the process, only those DQ α alleles that are sufficiently well matched to the DNA sequences contained on the probes will remain attached to the probe strip. The amplified DQ α DNA retained on the strip is then visualized by color development in the dots contained on the DQ Alpha test strip. The DNA analyst interprets the DQ Alpha test results by reading the pattern of blue dots on the probe strips in order to determine which DQ α alleles are present in the DNA sample being tested.⁵

An important feature of the DQ Alpha typing kit is the control (or "C") dot placed on the probe strip. The C dot serves two functions. First, the C dot indicates whether adequate amplification and typing of the DQ α alleles has been achieved in a given test. Second, the C dot guides the DNA analyst in the typing

⁵ DNA analysts should interpret DQ Alpha results by reading the freshly developed test strip. The analyst should also take photographs that are of large enough size and sufficient clarity to be examined for subsequent interpretation and maintain such photographs as a permanent part of the case file.

of DNA samples potentially complicated by a mixture of DNA from more than one donor or containing DQ α subtypes not identified by the test strip. The manufacturer of the DQ Alpha testing kit designed the C dot to be the weakest spot on the strip in terms of visualization, thereby providing a threshold for the interpretation of the allelic dots on the DQ Alpha test strip. If, after the washing process the C dot is not visible, then no results on the test strip should be interpreted and typed because the results obtained on the allelic dots are below the threshold for reliable interpretation. Moreover, the DNA analyst should interpret those dots exhibiting a signal intensity that is less than the C dot with caution because this might indicate the presence of a mixed DNA sample, a procedural error such as improper washing, cross-hybridization, or contamination of the DNA sample.

In addition, controls such as a reagent blank, a negative DNA control, and a positive control must be included with each assay of the DQ Alpha test strips. The reagent blank is a check for possible contamination of the sample preparation reagents by other human DNA or by amplified DQ α DNA. The reagent blank is performed by carrying out the DNA extraction in a tube containing no sample. This reagent blank extract is then amplified and typed along with the test samples. The negative control is a check for contamination during the set up of the PCR reaction. If typing signals appear in the negative control, every effort should be made to locate the possible sources of contamination. Under no circumstances should the reagent blank control or the negative control show a positive signal. If these controls happen to show a positive signal, the affected samples must be re-tested. Finally, a positive control is provided as part of the DQ Alpha test kit and should be used with each amplification and hybridization to demonstrate that the kit is performing properly. If the DQ Alpha type of the positive control is not correct, the DNA analyst should re-test all of the affected case samples. As a last step, the DNA analyst should take photographs of all wet strips of all samples tested, including the reagent blank, negative control, and positive control. These photographs should be maintained as a permanent part of each case file.

B. Polymarker

Following the release of the DQ Alpha typing kit, the AmpliType PM PCR Amplification and Typing Kit, also known as "Polymarker," was developed and released. The Polymarker test kit allows for the simultaneous amplification of five specific loci: Low Density Lipoprotein Receptor (LDLR), Glycophorin A (GYPA), Hemoglobin G Gammaglobin (HBGG), D7S8, and Group Specific

Component (GC).⁶ The Polymarker kit contained detection reagents and DNA test strips for typing the LDLR, GYPA, HBGG, D7S8, and GC loci by using the same reverse dot blot format and process as the DQ Alpha test process -- *i.e.*, PCR amplification, hybridization, washing, visualization, and interpretation.

Under appropriate hybridization conditions, amplified DNA products containing the alleles designated on the test strip will bind specifically to a particular dot on the Polymarker test strip. The AmpliType PM test system includes a standard probe dot (the "S" dot) that serves the identical quality control functions as the C dot on DQ Alpha typing strips. In reading a Polymarker test strip, a DNA analyst should not type any of the five loci on the test strip if the S dot is not visible and should interpret any dots on the test strip that are lighter in color than the S dot with caution.

C. D1S80

The D1S80 locus is found in the non-coding region of the first chromosome.⁷ Since it was first described in 1988, the D1S80 locus has been used in forensic analysis because it shows a very high degree of polymorphism. Most individuals have alleles at the D1S80 locus containing between 14 and 40 tandem repeats. The observed variability in the combination of alleles ("heterozygosity") at this locus has been reported to be as high as 87.6%. Due to the large number of alleles associated with the D1S80 typing system, it is highly discriminating and is frequently a more effective system for the analysis of mixed samples than the DQ Alpha or Polymarker systems.

A DNA analyst tests the number of tandem repeats possessed by an individual at the D1S80 locus by running PCR-amplified DNA products in a gel using an electrophoretic process. D1S80 allelic bands are then visualized and photographed. Similar to the RFLP process, larger fragments of DNA (those containing more tandem repeats) run slower through the gel and can be observed toward the top of the gel, while smaller fragments (those containing fewer tandem repeats) run more quickly through the gel. Sizing ladders are run

⁶ These genetic markers, as well DQ α , are inherited independently, thus allowing the genotype frequencies to be multiplied in order to determine the frequency of occurrence for a particular genetic profile in a specific population of humans.

⁷ More than 30% of the human genome is composed of repeating segments of DNA that seem to act as fillers or spacers between the coding regions of DNA on chromosomes. These repeating segments of DNA appear not to control any genetic function, they nevertheless they are an inherited component of an individual's genetic makeup.

on the gel to determine the alleles present in each sample. D1S80 alleles are expressed as the number of repeats in each DNA fragment. For example, one individual might be typed for D1S80 as 18,24 (18 repeats and 24 repeats) and another person as D1S80 type 22,31.

III. STRs

Short tandem repeats (STRs) are the genetic markers most widely used at the present time by crime laboratories to type biological evidence samples. STR technology is fast, sensitive, and highly discriminating.

In principle, STR markers are very similar to the DNA markers used in RFLP and D1S80 typing; they are DNA fragments composed of a number of DNA repeats that vary in fragment size from one person to the next. STR markers are particularly useful in typing biological samples that are old or degraded because the DNA fragments used in STR typing are relatively small compared with the DNA fragments analyzed in RFLP or D1S80 testing. STR typing incorporates the use of PCR amplification and, therefore, is very sensitive and capable of producing DNA typing results from small amounts of biological evidence.

During the PCR amplification process, fluorescent dyes are incorporated into the DNA fragments. After the PCR process, DNA fragments are transferred to a gel matrix and separated by size using electrophoresis. This electrophoretic step can be conducted on a variety of scientific instruments, such as a DNA sequencer. These instruments detect the DNA fragments through the use of the fluorescent dyes attached to the DNA fragments. The advantage of this fluorescent dye detection strategy is that it allows "multiplexing," which is a technique that simultaneously detects multiple STR loci in a single analysis. Because multiplexing allows for several STR loci to be analyzed in a single tube, STR technology is considered a relatively fast method that delivers very discriminating results.

As the DNA fragments migrate through the gel matrix and into the instrument's detection window, the fluorescent tags attached to the fragments give off a signal that is captured by the instrument as a "peak" detected at a particular point in time in the analysis. Through the application of sophisticated software, the instrument is capable of converting the time the DNA peak was detected into first a fragment size and then a DNA type. This typing information can be recorded on a printout known as an "electropherogram." Similar to the results generated by D1S80 testing, allelic types developed through STR analysis typically are expressed as numbers. For example, at the "D3" STR locus, an

individual's DNA sample could be typed as a D3 type "16,18." The analyst then compiles all of the allelic typing information developed at multiple STR loci to produce a complete STR profile of known reference samples as well as the evidence samples.

Because STR typing is based upon a PCR platform, it is essential that DNA analysts handle all biological samples appropriately so that the chance of sample mix-up or contamination is minimized and that reliable and accurate STR results are obtained. In part, this means that appropriate positive and negative controls must be used throughout the STR typing process. DNA analysts must monitor the performance of all controls to minimize the chance of error and to assess the testing process. In addition to the use of positive and negative controls, the analyst should take advantage of features that are engineered into the STR reagent kits. For example, when used in conjunction with each other, the Profiler Plus and COfiler reagent kits used by forensic laboratories have a built-in redundancy at three loci -- D3S1358 ("D3"), D7S820 ("D7"), and amelogenin.⁸ The presence of these redundant STR loci is a control to detect possible sample switches or poor sample quality. If the typing results at the three redundant loci obtained by both the "Profiler Plus" and "Cofiler" kits are not concordant, the analyst should be alerted to a problem that must be resolved.

⁸ The COfiler and Profiler Plus STR kits are proprietary products of Applied Biosystems.

Appendix D

Acronyms

AE	absorption elution
AI	absorption inhibition
ASCLD	American Society of Crime Laboratory Directors
ASCLD/LAB	American Society of Crime Laboratory Directors/Laboratory Accreditation Board
City	The City of Houston, Texas
CODIS	Combined DNA Index System
DNA	deoxyribonucleic acid
DPS	Department of Public Safety
FBI	Federal Bureau of Investigation
FPIA	fluorescence polarization immunoassay
FTIR	Fourier Transform Infrared
GC	gas chromatography
GHB	Gamma Hydroxybutyrate
GRC	General Rifling Characteristic
GSR	gun shot residue
HLA	human leukocyte antigen
HPD	Houston Police Department
MS	mass spectrometry
MSP	Michigan State Police
NIBIN	National Integrated Ballistics Information Network
PCP	Phencyclidine
PCR	polymerase chain reaction
PDR	Physician's Desk Reference
PwC	PricewaterhouseCoopers LLP
QA/QC	quality assurance/quality control
RFLP	restriction fragment length polymorphisms
RFP	Request for Proposals
SOP	standard operating procedure
STR	short tandem repeats
TLC	thin-layer chromatography
UV	ultraviolet

1 TRIAL COURT CAUSE NO. 1384794
2 THE STATE OF TEXAS) IN THE DISTRICT COURT
3 VS.) HARRIS COUNTY, TEXAS
4 OBEL CRUZ-GARCIA) 337TH JUDICIAL DISTRICT

5 I, Mary Ann Rodriguez, Official Court Reporter in
6 and for the 337th District Court of Harris County, State
7 of Texas, do hereby certify that the following exhibits
8 constitute true and complete duplicates of the original
9 exhibits, excluding physical evidence, offered into
10 evidence during the jury trial in the above-entitled and
11 numbered cause as set out herein before the Honorable
12 Renee Magee, Judge of the 337th of Harris County, Texas,
13 and a jury trial beginning the 3rd day of June, 2013.

14 WITNESS MY OFFICIAL HAND this the 16th day of
15 October, 2013.

16
17
18
19 /s/ Mary Ann Rodriguez
20 Mary Ann Rodriguez, Texas CSR 3047
Expiration Date: 12/31/2013
21 Official Court Reporter
337th Court
22 1201 Franklin
Houston, Texas 77002
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24
25